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PERSPECTIVES IN RENAL MEDICINE

Vascular calcification: A stiff challenge for the nephrologist

Does preventing bone disease cause arterial disease?

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Vascular calcification: A stiff challenge for the nephrologists—Does preventing bone disease cause arterial disease? There has been an explosion of interest in vascular calcification in the last 5 years. Four key "germinal" findings have fallen onto very fertile soil. First, on the background of an increasing cardiovascular disease burden it has been found that at least cross-sectionally, and in a limited fashion prospectively, achieved dialysis plasma phosphate levels are linked to all-cause and cardiovascular mortality. Second, there are increasing reports of calcific uremic arteriolopathy in Australia and the United States. Third, we know know that the mechanical properties of the carotid artery, and the aorta, have a profound influence on survival for dialysis patients. Vascular calcification itself (as assessed by x-ray films and ultrasound) has been linked to aortic stiffness. Fourth, increasing numbers of studies are showing extremely extensive coronary artery calcification (CAC) in dialysis patients, even at a young age. From these apparently unlinked observations the following assertion has been posited—that in the widespread (over) use of calcium-containing oral phosphate binders (OPB) to prevent uremic osteodystrophy in our dialysis population we have unwittingly accelerated widespread uremic vasculopathy and thereby contributed to premature cardiovascular mortality.

It is the purpose of this article to discuss vascular calcification (and particularly CAC) in dialysis patients as we understand it today. We will review the published series, with special reference to the Sevelamer Treat to Goal trial and also discuss the new Kidney Disease Outcome Quality Initiative (K-DOQI) guidelines on the use of phosphate binders in chronic kidney disease.

There has been an explosion of interest in vascular calcification in the last 5 years. At the 2002 and 2003 American Society of Nephrology (ASN) meetings, and in the recent World Congress in Berlin, there were dozens of abstracts, and several sessions, where these issues were presented and debated. A few years ago we doubt whether the abstracts would have been accepted, or the sessions been planned. What has happened—why this sudden interest in chalky arteries?

Every so often, there is either a new idea, or, more frequently, a new way at looking at an old problem. Four key "germinal" findings have fallen onto very fertile soil. First, on the background of an increasing cardiovascular disease burden it has been found that at least cross-sectionally, and in a limited fashion prospectively, achieved dialysis plasma phosphate levels are linked to all-cause and cardiovascular mortality [1, 2]. This effect persists in large datasets [e.g., United States Renal Data System (USRDS)] even after allowance has been made for a variety of potential confounders.

Second, there are increasing reports of calcific uremic arteriolopathy in Australia [3] and the United States [4, 5]. This once very rare condition is now being seen as a necrotizing panniculitis or ascending acral gangrene typically in obese white diabetic women, some on warfarin [6] (see later).

Third, we now know that the mechanical properties of the carotid artery, and the aorta, have a profound influence on survival for dialysis patients [7, 8]. Vascular calcification itself (as assessed by x-ray films and ultrasound) has been linked to aortic stiffness, and to patient survival [9–11] and one possible determinant of arterial calcification is thought to be ingestion of calcium-containing oral phosphate binders (OPB).

Fourth, increasing numbers of studies are showing extremely extensive coronary artery calcification (CAC) in dialysis patients, even at a young age [12, 13]. Here again, in some studies a link has been reported between the ingested dose of calcium-containing OPB and CAC [13–15]. It has recently been shown that progressive CAC on continued calcium salt administration can be largely abolished by the use of sevelamer (Renagel) in place of calcium-containing OPBs [14].

From these apparently unlinked observations the following assertion has been posited—that in the widespread (over) use of calcium-containing OPB to prevent uremic osteodystrophy in our dialysis population we have unwittingly accelerated widespread uremic

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vasculopathy and thereby contributed to premature cardiovascular mortality. We have put bones ahead of vessels. A broken bone is painful and debilitating, but rarely fatal. With "broken" coronary arteries, or aorta, the implications are obvious.

We in the renal community are in the dock. The charges have been read out by the prosecution. To answer these charges we have to address a simple question—does oral consumption of calcium salts lead to vascular calcification and hence increased vascular stiffness in patients on dialysis? Although this is a "renal" question, nephrologists are not the only ones to have questioned a link between calcium intake and cardiovascular disease [16, 17].

How should we plead? Our defense should be based on a critical examination of the "evidence chain" that underlies the prosecution case. Will there be a "smoking gun" or is the prosecution case merely a bald and insubstantial narrative given passing verisimilitude by recourse to circumstantial evidence, fervor and commercial spin?

It is the purpose of this article to discuss vascular calcification (and particularly CAC) in dialysis patients as we understand it today. We will review the published series, discuss the pivotal sevelamer Treat to Goal trial in detail [14], report important information on CAC and vascular calcification, answer the charges as stated, and review the options for therapeutic change. The new Kidney Disease Outcome Quality Initiative (K-DOQI) guidelines on the use of phosphate binders in chronic kidney disease will also be discussed.

RISK-BENEFIT RATIOS FOR INTERVENTIONS

As a prelude we can remind ourselves that all treatments incur risk, and we hope that the benefit that also accrues will outweigh this risk. Very often we set out with one "care paradigm" aimed at one problem, only to find that in applying that paradigm we unwittingly have created something worse. The irony here is that with the widespread and effective use of aluminum-based OPBs to counter "renal osteodystrophy" (mainly hyperparathyroidism) there was a large price to pay (allied to exposure to aluminum in dialysate) for a small number of patients. Thus there was a retreat from aluminum to calcium for phosphate binding. This also seemed sensible as it was perfectly possible to induce a negative calcium balance across dialysis, and osteopenia is a common finding in dialysis patients. But now some say that calcium is the aluminum de nos jours?

Although not in the scope of this article we should be mindful of other potential therapeutic pitfalls. For example, are we, by systematically "overloading" patients with intravenous iron in a short-sighted attempt to improve erythropoiesis, unwittingly increasing susceptibility to both infection and to oxidative stress and cardiovascular disease [18]? In the transplantation setting, is the chronic exposure to rejection-preventing calcineurin inhibitors leading to more chronic interstitial fibrosis and premature graft loss [19]? These worrying examples all have a common theme—short-term advantage, long-term adverse consequences.

Table 1. Demographic and therapeutic changes

Demographic changes	
More women, older more complex patients, more diabetics	
Therapeutic changes	
Water softening, deionization	1960s to 1980s
Abandonment of aluminum as an oral phosphate binders	1980 to 1985
Widespread use of calcium-containing oral phosphate binders	Late 1970s, early 1980s
Widespread use of vitamin D and its metabolites to treat renal osteodystrophy	Late 1970s
Declining use of bone biopsy/radiologic skeletal survey	1980s to present day
Increasing use of intact parathyroid hormone measurements noninvasively to assess bone health	1980s to present day
Increased numbers of patients dialysed, with a "one size fits all" approach to dialysate preparation	Progressive trend
Shorter hours—adequacy focused on low-molecular-weight solutes	1985 onward

MANY CHANGES IN PATIENTS AND DIALYSIS PRACTICE

Over the last three decades there have been many changes in the type of patient whom we treat, and what we have routinely done to these patients. He/she is likely to be older, with more comorbid conditions, cardiovascular and other, and more likely to have impaired glucose tolerance. These changes are listed in Table 1. We need to consider the integrated impact of all of these, and not to be selective. Older age (both "natural" and also progeria syndromes [20]), diabetes, and uremia are each associated with vascular calcification [21]. There are also sporadic or familial infantile vascular calcification conditions [22, 23]. Of course, as we shall see, vascular calcification is present even in young dialysis patients, and this requires explanation and interpretation.

Most body calcium, about 99.9%, is in the form of bone. The plasma calcium reflects body stores very badly. Plasma and intracellular calcium levels are kept tightly regulated, as many vital metabolic processes tolerate major departure from the normal range poorly. The skeleton is a highly effective buffer to keep plasma calcium in the normal range. Calciotropic hormones such as parathyroid hormone (PTH) and vitamin D play a pivotal role. It should be remembered though that there are PTH receptors in many tissues, including the vasculature. The same is true for the distribution of the vitamin D receptor [24]. Thus calcium and phosphate can be deposited into bone, or liberated from bone, as needed. Adjustments to ingested calcium can be made through the gastrointestinal

effects of vitamin D; renal excretion can also be minutely regulated. Self-evidently with renal disease and its treatment most of these important regulatory pathways are defective or disabled.

WHAT IS VASCULAR CALCIFICATION?

Contiguglia et al [25] showed that the calcium in arterial calcifications taken from vessels of uremic subjects was made up of hydroxyapatite crystals (the same form as is seen in the skeleton). Interestingly in calcified stenotic regions of arteriovenous fistulas (at sites of vessel wall injury) the material is a "brushite" form of calcium (magnesium) phosphate ("Whitlockite") which has been reported in the elastic lamina chiefly in the inner two thirds of the media of human aorta [26]. Calcium oxalate crystals are only relevant to systemic oxalosis, where vessel injury calcification and gangrene are well-described [27].

Calcification in the vessel wall occurs in two distinct sites. Atherosclerotic plaques as they mature and start to become "complex" typically have associated calcification. This is a hallmark of advancing atherosclerosis, and is seen in the aorta, coronary, and other muscular arteries. This process starts in childhood and adolescence [28]. The elastic lamina of large and medium-sized arteries can also calcify (particularly around fractured disorganized elastin fibers), this is "medial" calcification, responsible for "pipe-stem" or "tram-line" appearances (once known as Monckeberg's medial calcinosis [29]) as considerable lengths of the arterial tree calcify (Figs. 1 and 2). Both the giraffe and the African elephant also show this type of arterial pathology as they age [30]. This process was also observed in post-mortem histopathologic specimens from Egyptian mummies from the XXIst (circa 950 BC) dynasty (Jurgen Floege, personal communication). It would be a mistake not to consider these processes separately, as the vascular consequences (occlusion with atherosclerosis and vascular stiffening through medial calcification) are different. A recent report using plain x-ray films and ultrasound to delineate hemodialysis patients with intimal or medial femoral/carotid calcifications showed that the presence of any vascular calcification was associated with reduced survival—but that intimal lesions (seen in older patients) conferred a worse prognosis compared to medial calcification (seen in younger dialysis patients) [15]. It is likely that in some vessels (e.g., coronary arteries), intimal and medial vascular calcification, can colocalize. Interestingly, the promotors and risk factors for both types of vascular calcification though similar are not identical—unlike atheroscleoris, medial vascular calcification is not an inflammatory process per se [31].

Large elastic, medium-sized muscular arteries, and arterioles, can all calcify. Veins hardly ever undergo these changes [unless injured [32], or arterialized as after coronary artery bypass grafting (CABG) and arteriovenous fistula formation]. Patients with pulmonary hypertension can develop calcification in the pulmonary arterial tree [33].

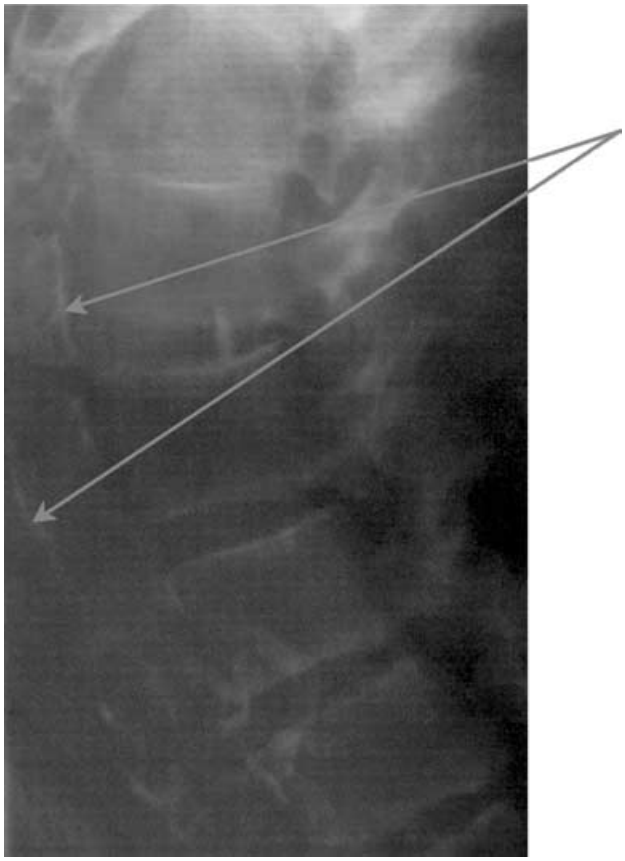

Fig. 1. Plain x-ray film showing tram-line medial calcification.

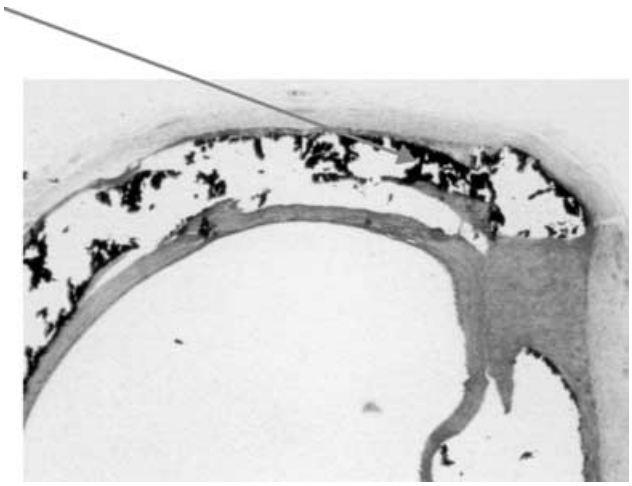

Fig. 2. Histology of medial calcification (courtesy of Dr. Sharon Moe, University of Indiana, Indianapolis, Indiana, USA).

For many years it was thought that it was physicochemical factors alone that regulated calcification (i.e.,

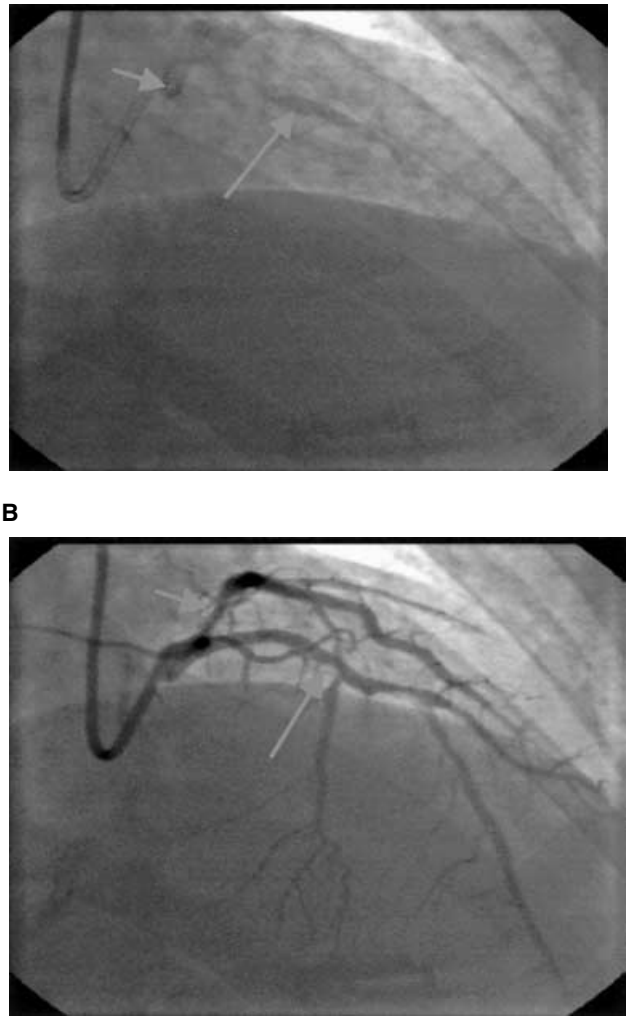

Fig. 3. Fluoroscopic calcification in coronary arteries—comparison with coronary angiogram.

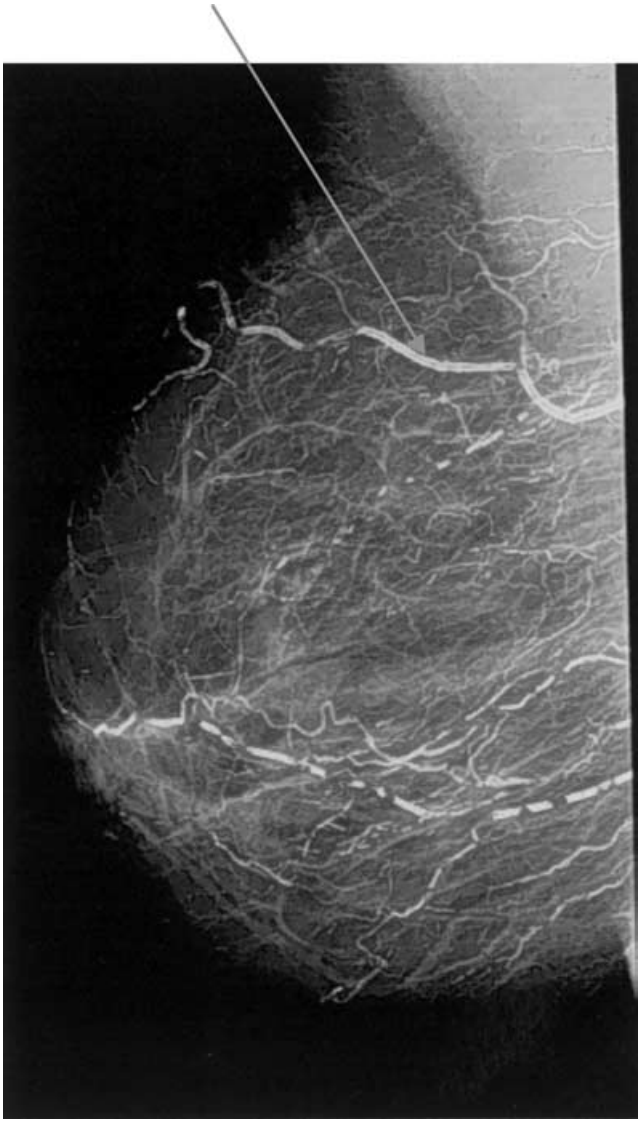

Fig. 4. Xerography (mammography) showing medial calcification in breast arteries.

calcium-phosphate product) and pH (alkaline pH favoring calcification) [34]. However, Virchow with typical lucidity and perception had pointed out in the 1850s the similarity between ossification and calcification [35], and ossification it had long been realized was a more elaborate regulated process involving the synthesis of a matrix which was then calcified. These seminal observations and deductions were destined for a long gestation.

HOW CAN WE DETECT, ASSESS, AND QUANTIFY VASCULAR CALCIFICATION?

The simplest technique is plain x-ray films. Pipe-stem calcification of the tunica media and internal elastic lamina produce spectacular films (Figs. 1 and 2). Often the patient has no, or nonspecific, symptoms however dramatic the x-ray films may appear. However, despite the simplicity of this technique it is both insensitive and difficult to quantify. Some degree of differentiation between intimal and medial calcification may be inferred from the use of plain films and ultrasound of the vessels to demonstrate patency and no luminal lesions [15]. Fluoroscopic appearances of valvular and coronary calcifications have been noted for decades (Fig. 3). Low-intensity ("xeroradiography") techniques such as mammography can show vascular calcification well in the context of soft tissue densities (Fig. 4). Vascular ultrasound can show calcified plaques, and calcified media, quite well. More recently, intravascular ultrasound and optical coherence tomography have been used, and these offer further refinements in sensitivity.

But of all these techniques it is computed tomography (CT) scanning (Fig. 5) that allows both detection and also quantification of the extent and the severity of vascular

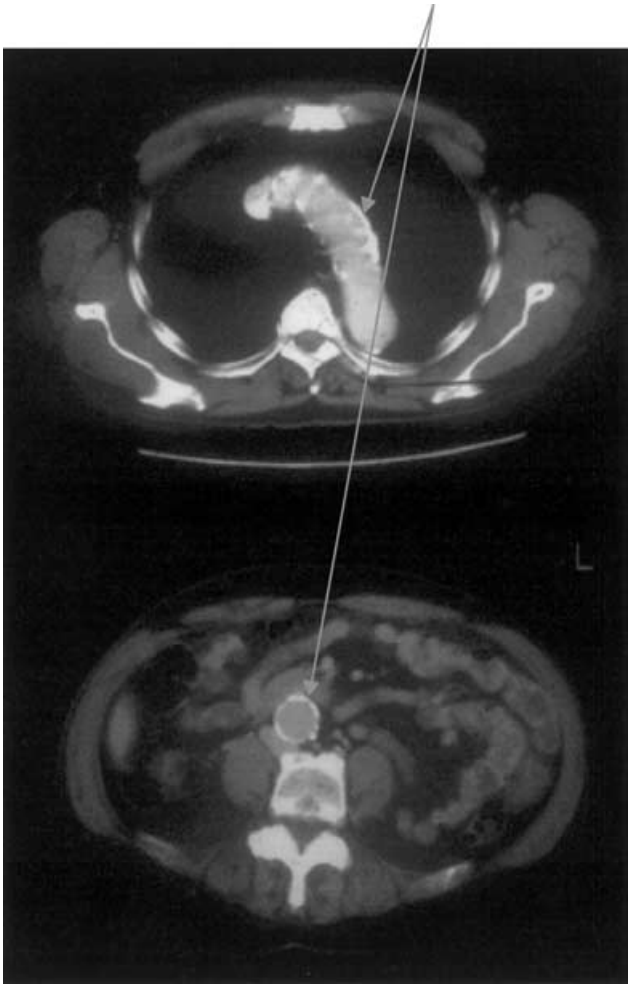

Fig. 5. Computed tomography (CT) scan of thoracic aorta showing extensive calcification.

calcification (though not a differentiation between intimal and medial calcium deposition). Electron-beam CT scanning with its rapid acquisition time (and relatively poor spatial resolution) has been developed with cardiac gating to examine coronary artery calcification (Fig. 6). Modern multislice/helical CT scanning machines now offer excellent spatial (but poorer temporal) resolution for large and medium sized arteries.

The origins of interest in this area were in histologic examination of postmortem arterial specimens. This remains the qualitative "gold standard." Quantification is very taxing.

There are other techniques that can show the presence of calcium, at microscopic level, but not establish its presence in vessels as opposed to a more diffuse interstitial deposition. These include ultrasonic/echocardiographic signal backscatter analysis [36], energy subtraction radiography [37], and nuclear medicine bone tracer techniques [38].

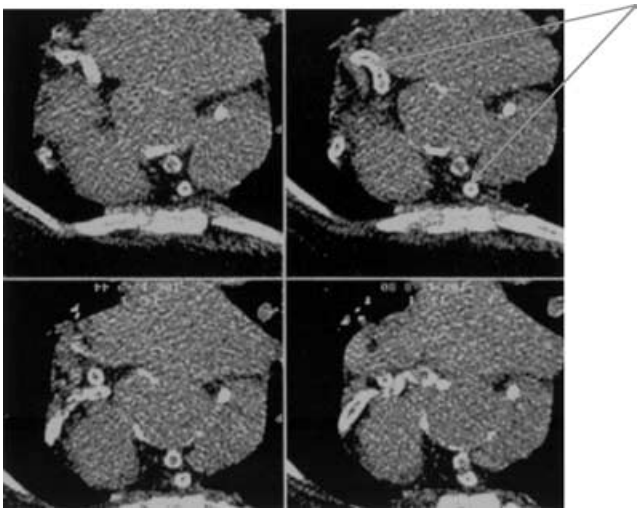

Fig. 6. Electron beam computed tomography (CT) scan showing severe calcification in coronary arteries.

BIOLOGY OF VASCULAR CALCIFICATION IN RENAL AND NONRENAL PATIENTS

Although vascular calcification has traditionally been considered to be a physicochemical "passive" precipitation in the tunica media of vessel walls, there are abundant animal and clinical data supporting the notion that more important than this passive process is an active ordered and regulated process. There are many complex bone-synthetic pathways (which closely resemble skeletal osteogenesis) in the vessel wall that involve a variety of genes and proteins with intimate involvement in mineral metabolism. This complexity is as true for calcification of complex atherosclerotic plaques as it is for medial arterial calcification. Various bone-related proteins such as osteonectin, osteopontin, PTH, PTH-related peptide, osteroprotegerin, and bone morphogenic protein can be found in atherosclerotic plaques and in sites of medial arterial calcification [39, 40].

The evidence that what is occurring in vessel walls is ectopic ossification comes first from clinical and histologic descriptions of structures resembling bone and even bone marrow, second that the form of calcium phosphate crystals found are hydroxyapatite (as in the skeleton), third matrix vesicles, which in bony ossification serve as the focus for ossification initiation, are also seen in vessel walls in proximity to calcified areas, and finally the immunocytochemical demonstration of a number of characteristic bone-related proteins such as collagen I and a number of noncollagenous bone matrix proteins.

In nephrology we have been interested in the relatively small amount of information that has come from short-term or cross-sectional human studies of patients on dialysis as a result of diverse renal pathologies. What is singularly lacking is any good evidence about the role of genetic factors as well as the obvious environmental ones.

Table 2. Main bone morphogenic proteins and noncollagenous bone matrix proteins and genes involved in vascular calcification in animals and humans

Protein	Function	Vascular calcification
Osteopontin	Antiapoptotic signal for osteoclasts; chemotactic factor for MC and MP	Recruitment of MP into area of vessel injury; osteopontin knockout mice develop stronger skeleton; osteopontin restrains mineralization
Osteoprotegerin/RANKL	Inhibitor of osteoclastogenesis; member of tumor necrosis factor-α receptor superfamily	OPG inhibited by cyclosporine A; OPG inhibited by osteopontin; restrains mineralization
Matrix GLA protein	Expressed in chondrocytes and vascular smooth muscle cells and increased expression in lipid-rich plaque	Knockout mice get dramatic early vascular calcification; warfarin effect on gamma-carboxylation (as osteocalcin); potent inhibitor of mineralization; inhibits bone morphogenic protein-2
Parathyroid hormone (PTH) recombinant protein	Paracrine factor with potential humoral activity in hypercalcemia of malignancy; shares PTH1 receptor with PTH; involved with normal bone physiology	Upregulated expression by vessel injury and angiotensin II; antagonizes procalcifying effect of vitamin D_3; down-regulates bone morphogenic protein-2
Bone morphogenic protein-2	Osteoblastic and mesenchymal differentiation	Promotes ossification
Bone morphogenic protein-7	Differentiation factor; normal tubular epithelial differentiation; osteoblastic differentiation	Null mouse has renal failure and agenesis; reverses heterotopic calcification in animal models
Vitamin D	Diverse functions through vitamin D receptor-dependent and independent pathways	Medial calcification; vascular smooth muscle cells have vitamin D receptors; raised calcium-phosphate product
Fetuin	Synthesized in liver; soluble transforming growth factor-β antagonist; negative acute phase reactant	Knockout mice get ubiquitous soft tissue and vascular calcification; potent inhibitor of mineralization

Abbreviations are: MC, monocyte; MP, macrophage; OPG, osteoprotegerin; RANKL, receptor activator of NF kappaB ligand.

This is worrying, as there are abundant data from animal models and from large clinical/epidemiologic databases that suggest genetic factors are intimately involved with the tendency to calcify.

There are several mouse models of accelerated vascular calcification—matrix GAL protein (MGP)-null, b-glucoronidase-deficient KLOTHO, carbonic anhydrase II-deficient, desmin-null, and osteoprotegerin-null—from which we can deduce that some genes are pivotal to the control of this process (reviewed in detail in [41]). It is not known (yet) whether the various forms of fetal and infantile accelerated calcification syndromes (both familial and sporadic) are human manifestations of single gene disorders [42]. In a recent report of a single case of a 25-month-old infant with advanced (and fatal) idiopathic infantile calcification syndrome there were deficiencies of extracellular pyrophosphate (PPi) and of PPi-generating nucleoside triphosphate pyrophosphohydrolase (NTPPPH) in his temporal artery lesions. Idiopathic infantile arterial calcification has now been studied in 11 unrelated kindreds [43], and mutations leading to inactivation of ecto-nucleotide pyrophosphate/phospodiesterase 1 (a cell surface enzyme which generates inorganic phosphate and serves as an essential physiologic inhibitor of calcification) have been shown, Plasma cell membrane glycoprotein-1 (PC-1) activity, associated in mice with arterial and periarticular calcification, tends to support this hypothesis [44]. Table 2 lists a selection of the gene and protein "major players." One genetic factor contributing to aortic calcification in the mouse is the Dyscalc locus, previously shown to contribute to myocardial calcification. The Dyscalc locus, on proximal mouse chromosome 7, segregated with vascular calcification in a large cross between susceptible strain DBA/2J and resistant strain C57BL/6J. Further evidence was observed by analysis of recombinant inbred strains derived from various susceptible and resistant parental strains [45].

Data derived from large clinical studies support the hypothesis that genetic/familial factors/polymorphisms have direct relevance. However, many studies are underpowered, and data from large populations are required. We lack such information about renal cohorts.

After adjusting for risk factors including age, gender, fasting glucose level, systolic blood pressure, pack-years of smoking, and low-density lipoprotein (LDL) cholesterol, 41.8% of the residual variation in CAC quantity in patients with coronary disease was attributable to genetic factors ($P = 0.0003$) in a large study (698 asymptomatic subjects) [46].

Members of 1256 Pima Indian nuclear families with 3339 offspring were examined radiologically for medial arterial calcification of the feet. Controlled for age, gender, diabetes, serum cholesterol, and blood pressure, offspring of one parent with medial arterial calcification (MAC) had 3.3 (95% CI 1.5 to 7.6) times the odds of MAC, as did offspring of parents without MAC while offspring with both parents affected had an even higher risk (odds ratio, 8.1; 95% CI 3.4 to 18.8). These findings suggested that the factors responsible for the familial clustering of MAC may be different from those for diabetes [47].

Finally, a large study examined CAC by electron beam CT in type 2 diabetics. Extent of CAC, adjusted for age, was positively associated with male sex ($P = 0.0003$), reduced high-density lipoprotein (HDL) ($P = 0.02$), albumin-to-creatinine ratio ($P = 0.008$), and cigarette pack-years ($P = 0.03$). CAC was also positively associated

with a history of angina, myocardial infarction, stroke, and vascular procedures (all $P < 0.01$). CAC, adjusted for age, gender, race, and diabetes status, was heritable ($h^2 = 0.50$; $P = 0.009$). In multivariate analysis with additional adjustment for HDL, body mass index (BMI), hypertension, and smoking, $h^2 = 0.40$ ($P = 0.038$). These results suggested that strong (independent) genetic factors as well as environmental factors contribute to the variance of CAC in individuals with type 2 diabetes [48].

Relationships between the E-selectin S128R polymorphism and CAC, a marker of atherosclerosis detected by electron beam CT, were examined in 294 asymptomatic women aged 40 to 88 years and 314 asymptomatic men aged 30 to 80 years from the Epidemiology of Coronary Artery Calcification Study. The E-selectin polymorphism was not associated with presence of CAC in men of any age or in women over age 50 years. In women 50 years of age or younger the E-selectin polymorphism was significantly associated with presence of CAC after adjustment for age, BMI, systolic blood pressure, ratio of total cholesterol to HDL cholesterol, and smoking [49].

The A-7 or Ala 83 alleles of the MGP gene may confer an increased risk of plaque calcification and myocardial infarction; however, the observed relationships were weak or limited to subgroups of patients and therefore need further confirmation [50].

A large autopsy series comprised 700 Caucasian Finnish men, aged 33 to 70 years (The Helsinki Sudden Death Study). Coronary stenosis and surface area of atherosclerotic changes (fatty streaks, fibrous plaques, complicated lesions, and calcification) were measured and the presence of myocardial infarction and coronary thrombosis recorded. Tumor necrosis factor (TNF)-α and TNF-β genotypes were determined. Men with TNF-α 22 or TNF-β 11 genotype tended to have more fibrous lesions and calcification in their coronary arteries [51].

Patients with coronary artery disease and the angiotensin-converting enzyme (ACE) DD genotype had a significantly higher incidence and greater extent of coronary lesion calcification, as determined by intravascular ultrasound (IVUS). This finding indicated that the ACE I/D gene polymorphism was related to the development or progression of atherosclerotic plaque calcification [52].

Thus far no study has attempted to examine any of the above polymorphisms, or the Vitamin-D receptor polymorphism, in the context of renal failure patients. Such work is required urgently.

Potentially important "leitmotiven" in this dense orchestral symphony include the potential for hyperphosphatemia (e.g., >1.4 mmol/L) to change the phenotype of human aortic vascular smooth muscle cells in vitro from contractile to secretory in a fashion dependent on normal sodium-phosphate cotransport, Pit1, leading to up-regulation of many genes associated with matrix mineralization [53].

Primary hyperparathyroidism has been increasingly recognized as a risk factor for cardiovascular disease—through calcification, hypertension, and left ventricular hypertrophy [54, 55]. Secondary (and tertiary) hyperparathyroidism has a long association with cardiovascular pathology. Indeed, PTH as a uremic toxin has a rich history [56]. Different studies at different times have linked mortality on dialysis to excess phosphate, excess calcium or PTH. In the study of Klassen et al [57] of the effect of pulse pressure on hemodialysis mortality, calcium levels did predict mortality on dialysis in a multivariate analysis of survival culling data from a 12-month period, but neither calcium, phosphate, calcium-phosphate, nor PTH affected the relationship between pulse pressure and mortality, which was much stronger. Interestingly, in nonrenal patients with controlled dyslipidemia it has recently been reported that increases in pulse pressure best predicted progressive vascular calcification of the aortic wall [58]. Thus, increased vascular stiffness begets calcification, which in turn begets increased vascular stiffness.

Vitamin D therapy has been recognized for decades as a potential vascular toxin, particularly in excess. Vitamin D has diverse, concentration-dependent, effects, via—but also independent of—the vitamin D receptor, promoting plaque calcification and atherosclerosis [59, 60]. Even in the earliest days of vitamin D usage (as an "extract" of ergosterol), its potent ability to "calcify" predominantly the medial layer of arteries was noted in animals [61, 62] and in humans [63, 64]. With pharmacologic doses of vitamin D there is often hypercalcemia, and elevated calcium-phosphate product, which per se can accelerate extraosseous calcification. The postmortem study by Milliner et al [65] of soft tissue calcifications in pediatric patients showed an association between vitamin D metabolite usage and vascular calcification (no association was found for calcium and phosphate). The plain x-ray study by Goldsmith et al [21] of vascular calcification in hemodialysis patients also showed an association between vitamin D metabolite levels and the extent and progression of vascular calcification. One mechanism that can be independent of any effect of vitamin D on plasma calcium has come from the work of Jono et al [66] where it has been shown that $1,25(OH)_2D_3$ exerts a stimulatory effect on vascular calcification through direct inhibition of the expression of PTH recombinant protein (PTHrP) in bovine vascular smooth muscle cells, as an endogenous inhibitor of vascular calcification. Moreover, the stimulatory effects of $1,25(OH)_2D_3$ on alkaline phosphatase activity and osteopontin expression may contribute to its promoting action in vascular calcification.

Further indirect support for a potentially important role for vitamin D metabolites in the genesis of cardiovascular disease comes from a recent retrospective report of increased mortality in North American hemodialysis patients taking calcitriol rather than paricalcitol [67].

It must be remembered, however, that in the general (nonuremic) population there may be a different relationship between calcification and vitamin D. Braam et al [68] has recently reported that there is a favorable effect on large artery hemodynamics from the administration of vitamins D and K.

Leptin (in excess in obesity, old age, type II diabetes uremia) has receptors on aortic vascular smooth muscle cells, and has been shown in vitro to promote vascular calcification of (bovine) vascular cells [69].

Oxidized lipids can induce inflammation and endothelial/vascular smooth muscle cells damage as an integral part of atherosclerosis (whose inflammatory—"atherosclerotic"—nature is of equal importance to its biologic behavior as is the more pedestrian lipid-accumulation facet). The inflammatory response to chemically altered lipid deposits involves monocyte-macrophage chemotaxis and differentiation—perhaps this atavistic response is useful in the context of lipid-rich bacterial cell membranes which stimulate the immune response appropriately? Certainly the calcification in the context of atherosclerosis can be regarded as inflammatory. Nitta et al [70] examined the expression of osteopontin in atherosclerotic aortas of hemodialysis patients and performed a prospective longitudinal study by using CT scans to detect aortic calcifications and by measuring the plasma osteopontin concentration by enzyme-linked immunosorbent assay (ELISA) in 36 hemodialysis patients and in 35 healthy volunteers. By immunohistochemical staining, osteopontin was abundantly localized in atherosclerotic plaques of hemodialysis patients. The macrophages surrounding the atheromatous plaques were identified as the osteopontin-expressing cells. The concentration of soluble plasma osteopontin was significantly higher in hemodialysis patients compared to concentrations in age-matched healthy volunteers (837.3 ± 443.2 ng/mL vs. 315.1 ± 117.4 ng/mL) ($P < 0.01$). The osteopontin concentration was positively correlated with the aortic calcification index in hemodialysis patients ($r = 0.749$, $P < 0.01$). However, the case for inflammation directly driving pure medial aortic vascular calcification may not be so clear-cut. Proudfoot and Shanahan [31] have emphasized the importance of apoptotic bodies in the elastin layer of the vessel media acting as crystallization nidus in medial calcification rather than the association of inflammatory cells with chemically altered lipids in intimal plaques as is seen in atherosclerosis [31].

It is important also to note that increased arterial calcification in the context of lower HDL—leaving unapposed the action of oxidized-LDL—has been reported in both renal and nonrenal contexts [71, 72]. Also, Parhami et al [73] have recently shown that when treated with HDL, alkaline phosphatase activity, a marker of osteogenic differentiation of osteoblastic cells, was significantly reduced in calcifying vascular cells. Prolonged treatment with HDL also inhibited calcification, further supporting the antiosteogenic differentiation property of HDL when applied to vascular cells. HDL inhibited the osteogenic activity that was induced by inflammatory cytokines interleukin (IL)-1β and IL-6 as well as by minimally oxidized LDL. HDL also partially inhibited the IL-6-induced activation of signal transducer and activator of transcription 3 in calcifying vascular cells, suggesting that HDL may inhibit cytokine-induced signal transduction pathways. The inhibitory effects of HDL were mimicked by lipids extracted from HDL but not by HDL-associated apolipoproteins or reconstituted HDL. Furthermore, oxidation of HDL rendered it pro-osteogenic. Taken together, these results suggest that HDL regulates the osteoblastic differentiation and calcification of vascular cells and that vascular calcification may be another target of HDL action in the artery wall.

The theme of manipulation of natural (circulating or local) inhibitors of the vascular calcification process (hydroxyapatite cystallization) is also relevant to the chronic inflammatory milieu of uremia, as both osteoprotegerin and alpha-2-Heremann Schmitt glycoprotein (AHSG), or human fetuin levels decline with increasing inflammation [74]. This group has recently reported an elegant series of experiments and observations on a cross-sectional study of 312 stable patients on hemodialysis to analyze the inter-relation of AHSG and C-reactive protein (CRP) and their predictive effect on all-cause and cardiovascular mortality over a period of 32 months. The capacity of serum to inhibit $CaxPO_4$ precipitation was tested in patients on long-term dialysis ($N = 17$) with apparent soft-tissue calcifications, and in those on short-term dialysis ($N = 8$) without evidence of calcifications and cardiovascular disease. AHSG concentrations in serum were significantly lower in patients on hemodialysis [mean 0.66 g/L (SD 0.28)] than in healthy controls [0.72 (0.19)]. Low concentrations of the glycoprotein were associated with raised amounts of CRP and with enhanced cardiovascular ($P = 0.031$) and all-cause mortality ($P = 0.0013$). Sera from patients on long-term dialysis with low AHSG concentrations showed impaired ex vivo capacity to inhibit $CaxPO_4$ precipitation. Reconstitution of sera with purified AHSG returned this impairment to normal. Fetuin deficiency appears to be associated with inflammation and may thus link vascular calcification to mortality in patients on dialysis [75, 76] and possibly also in other conditions associated with chronic inflammation. Links between vascular calcification and chronic inflammation/raised cytokine levels have been demonstrated for dialysis patients [77, 78].

The mechanism of action of human fetuin is now being unraveled. It has recently been shown to play an important role in the clearance of apoptotic cells by augmenting phagocytosis [79]. Apoptotic cell bodies are a possible

crystal forming nidus in medial arterial calcification. In rat serum fetuin can inhibit the precipitation of hydroxyapatite from supersaturated solutions of calcium and phosphate in vitro and this is accompanied by the formation of the fetuin-mineral complex (FMC), a high-molecular-weight complex of calcium phosphate mineral and the proteins fetuin and matrix Gla protein that was initially discovered in serum of rats treated with etidronate and appears to play a critical role in inhibiting calcification in vivo [80]. There may be yet other fetuin-like compounds. In a recent study a 24 kD protein was found, similar in domain structure to fetuin and, like fetuin and MGP, containing several residues of phosphoserine and accumulating in bone. Exogenous spp24 associated strongly with FMC when added to serum containing it. These observations suggest that spp24 may, like fetuin and MGP, play a role in inhibiting calcification [81].

MGP is expressed exclusively in mice in the growth plate chondrocytes and in vascular smooth muscle cells. It is a tissue-bound inhibitor of calcification, as opposed to soluble fetuin. Mice with inactivating mutations in both alleles of the MGP encoding gene prematurely calcify the growth plate cartilage leading to skeletal deformity and osteoporosis. MGP knockout mice show extreme generalized medial arterial calcification and die from vascular disease prematurely (reviewed in detail in [41] and in [82]).

Core-binding factor alpha(1) (Cbfa1) is an essential transcription factor for osteoblastic differentiation and osteogenesis. Bone morphogenetic protein (BMP) is also a powerful inducer of differentiation of pluripotent mesenchymal cells to osteoblast lineage and bone formation. Recent studies suggest that Cbfa1 plays a critical role during BMP-induced osteoblastic differentiation through association with cytoplasmic BMP signaling molecules, Smads [83]. Moe et al [84] examined sections of the inferior epigastric artery from uremic patients for the presence of Cbfa1 and type I collagen and osteopontin by in situ hybridization and immunostaining. Also examined was the effect of pooled uremic sera from dialysis patients on the expression of Cbfa1 in bovine vascular smooth muscle cells in vitro. Cbfa1 and osteopontin were expressed in both the media and the intima in vessels that were calcified, but there was only minimal staining in noncalcified vessels. In vitro studies demonstrated that pooled uremic serum, compared to pooled control human serum, induced the expression of Cbfa1 in bovine vascular smooth muscle cells in a time-dependent, nonphosphorus-mediated mechanism. These results suggest that Cbfa1 is a key regulatory factor in the vascular calcification observed in dialysis patients and is upregulated in response to many uremic toxins.

Of so far theoretic importance in humans is that fact that the gamma-carboxylation of MGP is a vitamin K-dependent process that is inhibited by warfarin. Warfarin in rats leads to calcification of heart valves and the epiphyseal growth plate cartilage. Whether this is relevant to humans is controversial. Some have linked long-term warfarin usage to the increasing number of cases of calcific uremic arteriolopathy (Seyle's calciphylaxis) [82, 85]; however, there are no data to support an association between large artery calcification and use of warfarin in humans.

One of the most important factors in the pathologic processes that lead to ectopic calcification is the calcifying vascular cells (CVCs). The presence of osteocalcin—pathognomonic of osteoblastic terminal differentiation—suggests that central to this complex interplay resides a cell with an osteoblastic phenotype. Demer, Tintut, and Perhami [82] and others have derived primary vascular smooth muscle cell cultures from explants of medial tissue from normal and diseased blood vessels. These cells adopt a calcifying phenotype which includes synthesis of a plethora of bone-regulating proteins, either spontaneously or after manipulation of the culture conditions. The origins of these CVCs is still not clear, possibly derived from common marrow mesenchymal cells, as either vascular smooth muscle cells, in the vessel media, or adventitial pericytes (for detailed review see [82]).

OTHER POTENTIALLY RELEVANT CONTRIBUTORS TO THE PROCESS OF VASCULAR CALCIFICATION

Cases of aggressive systemic calcinosis, calcific uremic arteriolopathy, and arterial calcification have not infrequently been reported after successful renal transplantation [86–89]. While post-transplantation hypercalcemia and hyperparathyroidism, two "dialysis" calcifying promoters may very rarely persist in the engrafted patient, hypophosphatemia (and therefore normal or often low calcium-phosphate product) is the norm after renal transplantation. Similarly the use of vitamin D and phosphate binders of any sort after renal transplantation is very unusual. So other promotors of calcification need to be identified in this clinical settings (e.g., dyslipidemia which is typical after successful renal transplantation) with drugs like cyclosporine A and sirolimus contributing.

IS THERE A PLAUSIBLE BIOLOGIC RATIONALE FOR THE PRESENCE OF VASCULAR CALCIFICATION?

It can legitimately be asked what is the "function" of vessel calcification? One plausible explanation is that it is a manifestation of a repair response to vessel injury. Uremia per se causes vessel injury (most notably at endothelial cell layer level) and perhaps this repair process is distorted by uremia and by calcium-phosphate derangement? One fascinating theory compares the constant

remodeling of the bony skeleton to stress and strain (e.g., the increase in bone mass on exercise), and its reduction in bed-bound patients or weightless astronauts, in which response there is an early increase in intracellular calcium mediated by stretch-activated cationic channels (SA-CAT) leading to changes in cytoskeletal architecture, up-regulation of anabolic bone activity manifested by osteopontin, and increased mineralization in vitro [41]. One could speculate therefore that in response to increased cyclical shear-stress strain in the vessel wall of large arteries there would be fragmentation and fracturing of elastin fibres in the tunica media, to which resident "CVCs" would respond by (medial) mineralization [90, 91] catalyzed by "uremic factors" (e.g., phosphate, dyslipidemia, oxidative stress, lack of inhibitors of calcification) [84]. This speculation would fit beautifully with clinical observations about the patterns and onset of vascular calcification and functional vessel changes in uremic subjects.

WHAT ARE THE IMPLICATIONS OF VASCULAR CALCIFICATION FOR RENAL AND OTHER PATIENTS?

Vascular calcification in the media of blood vessels has been linked to increased stiffness of large conduit arteries in renal patients [11]. This reduced vascular compliance leads to increased pulse pressure, downstream barotrauma, reduced coronary perfusion, abnormal autonomic and endothelial vasomotor function. In addition, difficulty forming vascular anastomoses (arteriovenous fistula, CABG) or performing successful coronary arterial interventions are described (angioplasty, stenting), as are calcific emboli from heart valves and aortic plaques.

Fluoroscopic calcification at coronary angiography was shown to have a sensitivity of 78% and a specificity of 66% for the presence of a 50% or greater stenosis in one or more coronary vessels in 86 dialysis patients by Marwick et al [92]. In a recent electron beam CT-based coronary angiographic comparison study of 282 patients (101 type 2 diabetics of whom 89 had coronary artery disease) it was shown that electron beam CT scores for coronary calcification had a similar sensitivity and specificity for coronary artery disease comparing the diabetic and nondiabetic cohorts [93].

In the nonuremic clinical setting calcium in an atherosclerotic plaque is a marker of a complex arterial lesion. As such use has been made of the ability to detect vessel calcification as a marker for atherosclerosis, often in asymptomatic individuals [94]. It has recently been shown that high coronary artery calcium scores posed an extremely elevated risk (higher than that for abnormal nuclear medicine perfusion scans) for hard end point events (major coronary events) [95, 96]. This additional cardiac risk was also largely independent of more traditional cardiovascular risk factors.

However, it is known that in uremia atherosclerotic plaque is already calcified at a much earlier developmental stage (at a point where luminal encroachment is minimal) than is seen in nonuremic subjects' vessels [97]. Schwarz et al [98] showed that coronary plaques in patients with end-stage renal failure were characterized by increased media thickness and marked calcification, but not the size of the plaque. It is important therefore to approach the question of the short- and long-term implications to uremic patients of the presence of vascular calcification with an open mind.

In uremic subjects where there is so much (more) calcium in vessel walls (intima and media) there may be "stabilization" of arterial plaque lesions with the artery "held open," the calcium acting as it were as "nature's stent." From a biomechanical perspective motion and stress are reduced in a calcified arterial segment but an adjacent noncalcified segment (in practice at the beginning and the end of the cylinder of calcification) may paradoxically experience more wall stress. Ex vivo analysis of calcified plaques have suggested increased resistance to rupture. Recent papers have challenged the classical construct, which appears valid in nonuremic subjects, that atherosclerotic plaques containing calcium may engender plaque instability and promote potential rupture [99]. Thus, in uremia, arterial disease progression is less toward acute plaque rupture and more toward chronic ischemia and fibrosis through progressive luminal obliteration (contributing to myocardial ischemia, fibrosis, myocardial electrical inhomogeneity, arrhythmia, and sudden death) and increased vessel stiffness which in the aorta leads to increased left ventricular mass. Indeed, acute coronary syndromes though two to three times more common than in age-matched general populations, are much less amplified by uremia compared to 100-fold increases in sudden death rates, and 20-fold increases in mortality due to heart failure/left ventricular decompensation (Data from UK registries and USRDS). The prognostic importance of coronary artery calcification in uremic subjects is not (yet) known but is a focus of intense interest.

Nevertheless, overall, the presence and severity of calcification (a composite score taken from ultrasounds and plain x-ray films of the aorta and carotids) was linked in a recent series to aortic stiffness, and to patient survival for a French hemodialysis cohort [11].

POTENTIAL THERAPIES AND REMEDIES FOR VASCULAR CALCIFICATION: ANIMAL CLUES

There are only a few animal studies investigating agents to retard or to regress vascular calcification. From these, some potentially useful interventions that could be tried

in man include the use of bisphosphonates [100], calcium channel blockers [101], and aluminum-tanning pretreatment of cardiac valves [102, 103]. As aluminum of course is notorious in disrupting osteogenesis, it should not be surprising that aluminum may inhibit ectopic calcification. But it raises the question of whether today's vascular calcification is a result of ceasing to use an inhibitor, or by starting to use a promotor (calcium), or both effects?

EARLY ACCOUNTS OF VASCULAR CALCIFICATION IN RENAL PATIENTS: ROLE OF UNBUFFERED CALCIUM AND VITAMIN D—IS THIS "EPIDEMIC" OF VASCULAR CALCIFICATION A NEW PHENOMENON?

There is much interest in vascular calcification presently. But though the interest is new, and we know that vascular calcification is very widespread in renal cohorts, it is new, or just newly discovered, and how does it relate to the epidemic of cardiovascular disease seen in dialysis patients [104]?

Calcific uremic arteriolopathy has a long history, stretching back 100 or more years, to an original description in a medical report at Guy's Hospital [105], and, depending on one's prejudice, to Herod the Great (Donald Sherrard, personal communication). Large case series were reported at the beginning of the dialysis era in the 1960s and 1970s [4, 106, 107]. Similarly, as we have indicated vascular calcification in renal failure is not per se news. Re-reading some of these "old" cases dating from 1855 to 1945 (88 in number and beautifully reviewed by Mulligan in 1949 [34]) show that they were a mixture of osteolytic metastases, primary hyperparathyroidism, and parathyroid carcinoma and vitamin D toxicity. In nearly all of these cases, significant hypercalcemia was a cardinal feature.

In a clinically fairly pure cohort of long-hours dialysis, patients with modest and well-defined use of vitamin D and calcium [21] showed an association between measured $1,25(OH)_2D_3$ levels and the extent and progression of vascular calcification.

Can we be sure that the present vascular calcification "epidemic" is new? May it not be that much greater awareness of vascular calcification as an issue, and better screening and imaging analytic techniques, have recently helped us better to define a long-standing problem?

Autopsy studies–derived evidence

In the predialysis era Pollak et al [108] reported the presence of vascular calcification in 18/29 patients with secondary hyperparathyroidism. In 1969 Parfitt [109] (then at Cedars Sinai Los Angeles) in reviewing "soft tissue" calcification reported 8/16 dialysis patients with "arterial calcifications." An autopsy study from the period 1966 to 1975 of 56 dialyzed and 18 nondialyzed chronic renal failure patients reported 44/56 dialyzed patients but only 8/18 chronic renal failure patients had visceral (including myocardial) calcification. Interestingly, the correlation in this study between severity and anatomic extent of calcification with bone biopsy findings, and parathyroid gland weight and histology, was poor [110]. Terman et al [111] reported six autopsy cases of chronic dialysis patients who demonstrated myocardial, cardiac conducting system, and intense arterial calcifications (internal elastic lamina, and medial) with little or no atherosclerotic coronary disease. Ibels et al [112] in 1979 reported 18 autopsies on chronic dialysis patients only one of whom had angina. They found 92% patients with intimal thickening, 67% calcification, and abnormal internal elastic laminae in 83% compared to control autopsy specimens. Milliner et al [65] reported on 120 children who had died at a mean age of about 10 years with uremia, dialysis or after transplantation between 1960 and 1983 in Los Angeles; 29 had cardiac and 12 had coronary artery calcifications.

X-ray studies–derived evidence

Meema, Oreopoulos, and de Veber [113] from Toronto, Canada reported in 1976 an x-ray study of 364 skeletal surveys in 152 dialysis patients. 30% of 15 to 30 year olds and 50% of 40 to 50 year olds had vascular calcification (most commonly medial vascular calcification at the ankle). Progression of anatomical extent and severity was most marked in the nondialyzed group and interestingly was least in the hemodialyzed group. The same group reported in 1986 [114] and 1987 [115] on factors associated with progression and regression of x-ray film disclosed vascular calcification in chronic continuous ambulatory peritoneal dialysis (CAPD) patients between 1980 and 1985; all had some calcification (very mild 58%, moderate to severe in 42%). Progression occurred in 57% and regression in 13%. Progression was more likely if the plasma magnesium level was low.

In the study by Andresen and Nielsen [116] published in 1982, the overall frequency of calcifications in vessels and soft tissue was 39% in 184 patients with chronic renal failure. Calcification was most pronounced in the larger vessels in nondialyzed and dialyzed patients and in small vessels in renal transplant recipients. In nondialyzed patients and in dialyzed patients at the start of dialysis treatment, calcifications were more commonly found in women while in renal transplant subjects a male preponderance was seen. Renal transplant recipients, who developed osteonecrosis or spontaneous fractures after transplantation, showed at the time of transplantation a higher frequency of calcifications compared to renal transplant recipients, who did not develop these complications. In all groups a significant, higher frequency of calcifications was found in patients with radiologic evidence

of bone resorption compared to patients without resorptive bone changes. Before and during chronic hemodialysis and following renal transplantation the frequency of calcifications increased, a process that occurred slowly in nondialyzed patients and in renal transplant recipients, whereas an accelerated increase occurred in the first 15 months of the dialysis period in hemodialyzed patients. The frequency of calcifications increased with increasing age not only in the aorta, but also in the other vessels.

The most careful follow-up analysis study was a derived-score x-ray series from Goldsmith et al [21] where 38 long-term (surviving 10 to 25 years on 24 hours/week hemodialysis) were reviewed with their annual skeletal survey and clinicobiochemical dossiers. This "skeletal" survey series was reanalyzed as a "vascular" survey series. This covered the period 1970 to 1994 and involved over 600 patient years of hemodialysis. Aortoiliac calcification was seen in 39% at the onset of dialysis, the risk increasing with age. By a mean of 16 years on dialysis 92% of patients had vascular calcification. Central/elastic arteries could calcify independently of smaller peripheral muscular ones. Patient age, dialysis vintage, pulse pressure, plasma phosphate, measured plasma vitamin D levels, and severe hyperparathyroidism [by intact PTH (iPTH)] were associated with heavier vascular calcification. Parathyroidectomy would reduce, or abolish, further calcium accumulation in the vascular tree.

Electron beam CT studies–derived evidence (see also below)

Electron beam CT–derived CAC scoring has shown for adult patients already established on dialysis an 80% to 100% prevalence of CAC. This is lower in children, though in children on dialysis CAC progresses faster than in healthy adult patients (William Goodman, personal communication). Geoffrey Block has recently reported (personal communication) that 40% of incident dialysis patients, including a high percentage of diabetics just starting on hemodialysis, did not have CAC by electron beam CT; and only 17% had very heavy scores. This is contrasted with a recent predialysis study of diabetic patients where the vast majority had CAC evident and to a greater extent than nondiabetic controls [117], and which many other studies of chronic dialysis patients, where cross-sectionally, and with limited follow-up, there was near universal progression of CAC (see Table 4).

We can conclude that vascular calcification was a clear feature of patients well before calcium-containing OPBs were available, and that further calcium accumulation was, and is now, very common particularly once on dialysis. There are many closely-linked risk factors for the development and progression of vascular calcification, in diverse clinical situations.

Given the different patient groups, and the increasing use of much improved x-ray/electron beam CT techniques and scoring systems to detect vascular calcification, while it is certainly the case that vascular calcification is common if not ubiquitous, it is not possible to say with confidence that the epidemic of vascular calcification is "new." Table 3, compared with Table 1, serve to show how much of the present "evidence" is circumstantial and is capable of several interpretations.

ELECTRON BEAM CT STUDIES IN RENAL PATIENTS

In the general population this (screening) technology allows gated imaging of the heart and great vessels free from respiratory artifact by virtue of a 100 mseconds image acquisition window, compared to 300 to 500 mseconds time period needed for multislice CT machines. Calcium in the vessel shown by electron beam CT signifies a complex plaque reasonably accurately, compared with angiographic studies, and outcome data. As such use has been made of the ability to detect vessel calcification as a marker for atherosclerosis, often in asymptomatic individuals [94]. It has recently been shown that high coronary artery calcium scores posed an extremely elevated risk—and higher than that for abnormal nuclear medicine perfusion scans—for "hard events" (major adverse coronary events) [95, 96].

The first study to examine renal patients using this technology was by Braun et al published in 1996 [12]. In reality this was ahead of its time, and preceded general interest in this area. By the time of the second publication in 2000 by Goodman et al [13], in a different patient cohort and in a high-prestige journal, there was a much more receptive audience. Since then there have been several more full publications and abstracts [14, 71, 77, 78, 117–121]. These are summarized in Table 4 (full papers). Even so, to date there are only data around 500 patients, and only limited follow up on about 180 of these. In our opinion there is much more to be understood about what this technology is able to tell us.

SEVELAMER "TREAT TO GOAL" STUDY

In numerical terms this study, enrolling around 200 subjects, providing a baseline electron beam CT image in 186, and providing 12 months electron beam CT CAC and aortic calcification data on (just) 132 subjects is by far the largest study such involving dialysis patients [14]. The fact that this was in addition an intervention study, one of very few in the whole field of electron beam CT (in any patient grouping), is a cause for celebration for the investigators, who deserve congratulation for the organization of this undertaking. The virtual abolition of progression

Table 3. Possible confounding factors in the interpretation of mainly cross-sectional information about risk factors and vascular calcification

Risk factor	Association	Confounded by
High plasma phosphate	Phenotypic shift of contractile vascular smooth muscle cells to secretory CVC; mortality data	Is high phosphate a marker of severe bone disease, or noncompliance with dialysis, drugs, and diet?
High plasma calcium	Hypercalcemic episodes leading to direct precipitation	Hyperparathyroidism, vitamin D; lack of relation between calcium balance and blood calcium levels
High parathyroid hormone	Increases intracellular calcium levels	Use of more phosphate binders and vitamin D
Low parathyroid hormone	Lack of dynamic bone metabolic activity to buffer changes in plasma calcium	Previous use of vitamin D and phosphate binders; parathyroidectomy
Vitamin D therapy	Increases plasma calcium and phosphate levels; calcifies vessel wall tunica media; vitamin D receptors on vascular smooth muscle cells (especially with hyperphosphatemia, even if calcium normal); accelerates atherosclerosis	Hyperparathyroidism
Inflammation/redox stress	Reduces levels of alpha-2-Heremann Schmitt glycoprotein (fetuin) a naturally occurring inhibitor of calcification; accelerated atherosclerosis	Vascular disease may be the cause of inflammation
Warfarin	Inhibits matrix GLA protein; naturally occurring inhibitor of calcification	Often used in patients with poor hemodialysis access, including diabetics, older patients, and women; sharing risk factors for vascular disease
Dyslipidemia	Oxidized low-density lipoprotein causing endothelial/vascular smooth muscle cell damage; high-density lipoprotein protective against calcification	Hyperparathyroidism associated dyslipidemia; sevelamer reduces low-density lipoprotein cholesterol and raises high-density lipoprotein cholesterol
Avoidance of aluminum; high dose of oral calcium-containing phosphate binders	Positive calcium balance	High plasma phosphate levels; poor compliance with drugs, dialysis, and diet; alkalosis favors calcification (calcium carbonate/acetate); sevelamer leads to mild acidosis; aluminum is a potent inhibitor of calcification/ossification

CVC is calcifying vascular cells.

of aortic and coronary calcification over 24 months of follow-up in patients whose plasma phosphate was controlled by sevelamer, in contrast to the progression of vascular calcification seen in matched cohorts given calcium acetate or carbonate (Fig. 7)—despite the same calcium-phosphate product—is remarkable, and tells us that rapid progression of vascular calcification on (hemo)dialysis can be ameliorated. But crucially, it does not tell us how. The effects on the CAC score described in the study did not correlate with any other parameter (in part this may reflect the complexity of the scoring systems, and the need for more information about how best to analyze these rare types of electron beam CT-based progression studies).

Two recent studies do inform us to some degree about the significance of electron beam CT-derived CAC scores in chronic kidney disease patients. Haydar et al [122, 123] have published two studies, the first of which found a strong cross-sectional association between CAC and aortic pulse wave velocity (which measure of aortic stiffness has an impressive association with mortality on dialysis) [122]. In their second report, of even greater interest was a close association between the degree of coronary atherosclerosis and the extent of CAC, suggesting that there may be a similar relationship even in dialysis patients between extent of CAC and coronary atherosclerosis [123]. If this is confirmed, the implications of high electron beam CT scores, and the importance of preventing rapid progression of further calcification, are obvious.

The Treat to Goal Study cannot inform us solely about the role of oral calcium ingestion in the pathogenesis of cardiovascular disease. This is because the comparator drugs (sevelamer hydrochloride and calcium acetate/carbonate) differed not only in their "calcium loading/phosphate binding" capacities but also in an absolutely crucial area of relevance, namely, lipid-lowering capacity, with the sevelamer cohort showing around a 35% reduction in LDL cholesterol values [14]. Sevelamer (Renagel) is a hydrogel polymer (poly-allylamine hydrochloride) which among its properties can boast a bile-acid sequestrant effect. In a large study sevelamer reduced LDL cholesterol by 30%, and increased HDL cholesterol by 18%. Lipid profile changes of a similar magnitude were seen in the Treat to Goal Study [124].

The importance of this cholesterol modulation effect cannot be overstated as two recent trials in nonrenal cohorts have shown that substantial reductions in LDL cholesterol were associated with reduced progression of electron beam CT-measured CAC [72, 125]. From the second of these studies it was shown that in 32 patients with an LDL cholesterol level <100 mg/dL under treatment, the median relative change in CAC score was 27% during the untreated versus −3.4% during the treatment period ($P = 0.0001$). These data are consistent also with the small electron beam CT study by Tamashiro et al [71]

Table 4. Summary of coronary artery calcification (CAC) series (full papers) featuring renal patients

Author, year [reference]	CT method	Number age (range)	Patient types	Calcified	Score, range	Associations	Progression
Braun et al 1996 [12]	Electron beam	49 HD (28-74); 102 C (32-73)	HD (3-264 months dialysis) and CAD C	100% HD	4290 ± 1509 HD 406 ± 791 C	Hypertension; inverse relation with bone density	Rapid (arteries and valves) (incomplete)
Goodman et al 2000 [13]	Electron beam	39 HD (7-30); 60 C (20-30)	Young chronic HD	None <20 years; 14/16 >20 years; 3/60 C	1157 ± 1996 (C scores were 1, 2, 77)	Calcium intake; Ca x PO_4; longer dialysis	Rapid (incomplete)
Eifinger et al 2000 [118]	Electron beam	16 HD (14-39)	HD (13 previous RTx)	6/16	1-807	All asymptomatic	N/A
Tamashiro et al 2001 [71]	Electron beam	24 HD (53 ± 14)	HD (64 ± 69 months)	>90%	449 ± 605	CAD (SPECT, angiography)	Rapid if high-density lipoprotein low and TRIGS elevated
Raggi et al 2002 [119]	Electron beam	205 HD (57 ± 15)	HD (37 months) (17-63)	83%	595 (76-1000)	Age, diabetes more; female, black less; calcium, PO_4, and dialysis vintage	Baseline for Treat to Goal
Chertow et al 2002 [14]	Electron beam	186 imaged; 132 follow-up; 57 years (all >19)	HD (dialyzed about 3 years)	83% baseline	~650 (80-2300)	N/A	Reduced on sevelamer Treat to Goal
Oh et al 2002 [77]	ECG-gated spiral	39 (19-39)	13 HD (26 RTx)	92%	226 HD (205-293); 60 RTx (23-103)	Calcium PO_4; parathyroid hormone dialysis vintage; C-reactive protein; *Chlamydia*; homocysteine	N/A
Moe et al 2003 [120]	ECG-gated spiral	71 (49 ± 13 years)	33 HD (38 RTx)	70%	859 HD (0-8772); 314 RTx (0-4322)	Age, dialysis vintage	N/A
Merjanian et al 2003 [117]	Electron beam	32 DM RD (57 ± 1.5); 18 C DM (58.2 ± 1.9); 95 C non-DM (57 ± 0.9)	32 DM RD (DM 12.7 ± 1.1 years); 18 C DM (DM 10.1 ± 1.6 years	CAC 94%; AWC 66%; AVC 23%; MVC 25%; CAC 100%; AWC 22%; A, MVC 11%; AWC 59%; AWC 35%; AVC 6%; MVC 2%	C-C 55-789; CAC 12-202; CAC 0-90	Age, HTN, males, DM RD with CAC; age and DM RD with AWC; age, DM RD with AVC; DM RD with MVC	N/A
Stompor et al 2003 [78]	Multirow spiral	43 PD (50.6 ± 13.4 years)	43 PD (duration 2-96 months	CAC 53.8%	0-5502 Agatston units	Age, BMI, serum leptin. and C-reactive protein[a]	N/A
Sharples et al 2004 [121]	Electron beam	18 HD 54 (31-73)	27 (4-111 months duration)			Poor correlation with CAD	
Haydar et al 2004 [122,123]	Electron beam	50 patients	24 D (16 RTx); 10 predialysis	Almost all calcified	0-11,500 Agatston units	CAC strong association with aortic PWV and coronary atheroma	

Abbreviations are: HD, hemodialysis; PD, peritoneal dialysis; RTx, renal transplantation; DM RD, diabetic renal disease; AWC, aortic wall calcification; AVC, aortic valve calcification; MVC, mitral valve calcification; HTN, hypertension; BMI, body mass index; SPECT, single photon emission computed tomography; TRIG, triglycerides.

[a]These correlated with CAC but in multiple regression analysis only age was independently associated with CAC.

where more rapid CAC progression in dialysis patients occurred in those subjects with higher triglyceride, and lower HDL, levels. Obviously, the role of dyslipidemia in the etiopathogenesis of atherosclerotic plaques in blood vessels need not be reprised.

Finally, it must be said that there is in fact only one other study examining longitudinally the progression of electron beam CT CAC scores in the same patients. Here it was shown that the use of calcium channel blockers, as opposed to thiazide diuretics, in hypertensive

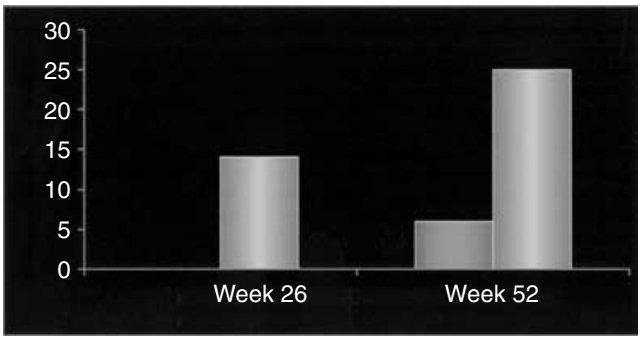

Fig. 7. Treatment effects of sevelamer (Renagel) on progression of coronary calcification in hemodialysis patients (comparator calcium containing oral phosphate binders) (from [14] with permission of the authors).

subjects was associated with lesser CAC progression [126].

We in the renal community, although fortunate indeed to have a CAC progression study involving an intervention, now urgently need a trial that compares statins, statins with calcium acetate, and sevelamer to reveal the relative contribution of lipid-lowering, avoidance of hypercalcemia, or other unknown effects. There is increasing evidence of multiple pleiotropic effects of sevelamer in humans, including alteration of lipid profiles, reduction in CRP, reduction in uric acid levels, and reduction in oxidative stress. Any or all of these alternative mechanisms, with avoidance of hypercalcemia, may be the explanation for the abolition of progression of vascular calcification. This is important, as there is no certainty that other noncalcium containing OPBs will have any of these additional, and possibly crucial, properties. Thus it is of great importance that, another nonaluminum, noncalcium phosphate binder such as ferric chloride, or the rare earth lanthanum chloride, should be compared to calcium acetate in the same way. Lanthanum chloride in vitro has anticalcification/antiatherosclerotic properties [127]. It would be important to see if these were found in human studies, despite the minimal absorption of lanthanum carbonate as a phosphate binder. Until we have this additional information either the lipid, or the calcium, explanations (or both) could be valid. Even the mooted larger long-term survival study in sevelamer vs. calcium acetate patients will not address this issue adequately, unless it can be controlled for cholesterol values, as cholesterol-reduction is now known to be associated with improved survival on dialysis [128].

KEY THINGS WE STILL NEED TO KNOW: BUILDING THE EVIDENCE CHAIN

In our view the evidence chain we have is incomplete. Among the things we need to understand better are (*1*) the relationship between CAC and aortic calcium scores, and vessel stiffness; (*2*) the origin(s) of vascular calcium (e.g., diet, dialysate, binders, bone) using mass balance methodology and with detailed analysis of bone turnover; (*3*) the importance to any success in impeding vascular calcification of aggressive lipid-lowering therapy and/or suppression of vascular inflammation over and above phosphate-reduction; (*4*) the complex relationship between plasma calcium and phosphate levels, vitamin D dose and levels, and the tendency to ectopic calcification; (*5*) the role of better dialysis strategies (e.g., daily or long-hours hemodialysis where control of plasma phosphate is vastly superior to conventional hemodialysis); and (*6*) Whether there is a special "sevelamer"-specific effect on vascular calcification that is not shared by other noncalcium-containing OPBs.

The importance of a full and proper understanding of the very complex inter-relationships between mineral metabolism and outcome is beautifully delineated by Stevens et al [129] in a recent longitudinal study of 515 Canadian dialysis patients. In this report it was shown that combinations of derangements of calcium, phosphate, and PTH were much more powerfully predictive than using individual parameters. Thus, the combinations of high calcium, phosphate, and high PTH, or low PTH, had the highest risks for mortality compared to normal calcium, phosphate, and high PTH, which had the lowest mortality.

When we do fully comprehend the above points, we will be nearer to understanding how (or even if) we should employ CT-based techniques to quantify vascular calcification extent and progression, or to screen for vascular disease. Such technologies may however allow for an accurate assessment of incremental calcification with time on dialysis.

THE NEW (2003) K-DOQI GUIDELINES ON PHOSPHATE BINDERS IN CHRONIC KIDNEY DISEASE

In the face of growing concerns about cardiovascular disease and vascular calcification, a careful process of evidence review and expert deliberation has resulted in new guidelines. In general these new recommendations necessarily come with "opinion" rather than with "evidence" labels. Very little indeed can be stated with confidence for predialysis patients, even when to start phosphate binders, with what binders, to what target phosphate levels? The following recommendations are for dialysis patients [130]: (*1*) initial therapy of raised phosphate levels [>1.8 mmol/L (5.5 mg/dL)] refractory to dialysis and diet can be started using either calcium or nonmetal salt-based phosphate binders; (*2*) use of a cocktail of oral phosphate binders is strongly encouraged, with a limit of 1.5 g of calcium salts (making a maximum total of 2 g of elemental calcium per day in conjunction

with dietary calcium intake); (*3*) calcium salts should be avoided in patients with sustained iPTH levels of <150 pg/mL, or plasma calcium levels of >2.55 mmol/L (10.2 mg/dL); vitamin D compounds should also be avoided or ceased with calcium levels of >2.55 mmol/L (10.2 mg/dL); (4) noncalcium-based binders should be preferred in patients with severe vascular or soft tissue calcifications; (*5*) plasma calcium should be maintained at the lower end of the normal range [2.1 to 2.35 mmol/L (8.4 to 9.5 mg/dL)]; and (*6*) calcium-phosphate product should be kept <4.4 $mmol^2/L^2$ (55 mg^2/mL^2) by focusing first on controlling plasma phosphate.

These guidelines suggest a radical shift away from calcium usage. In reality this will mandate the use of a variety of different phosphate binders in many patients if the central phosphate control targets are to be achieved. Thus patients on several, or all, of the older phosphate binders (calcium, magnesium, aluminum) as well as the new drugs (sevelamer, lanthanum, and iron compounds) will become much more common. The challenge to control phosphate (the primary goal) will remain a severe one. The balance of risk between achieving good phosphate control, versus avoiding excess calcium loading, needs to be determined, given that plasma phosphate (and emphatically not plasma calcium) has been linked to mortality on dialysis.

OUR CONCLUSIONS AND RECOMMENDATIONS

In our view the key issue that should concern us all is sustained and widespread oversuppression of hyperparathyroidism by engendering a net positive calcium balance for the patient often using both vitamin D and calcium-containing OPBs. Low-turnover bone states are the now the norm not only in dialysis patients but also in predialysis patients as shown by a recent bone-biopsy study [131]. This tendency is worsened by calibration of the use of vitamin D by hypercalcemia, rarely by use of vitamin D levels, and by infrequent plasma iPTH assays [at best an approximate guide to bone histology—the conceptual difference is in fact between a random glucose value (iPTH value) and glycated hemoglobin (bone biopsy)]. iPTH values are at their least clinically predictive over the range 100–400 pg/mL (which is where we try to engineer the majority of patients). Intact osteocalcin and bone-specific alkaline phosphatase may be more useful tools which should be tested prospectively in clinical trials [132, 133]. A whole paradigm shift in the treatment of hyperparathyroidism and ectopic calcification may well be close at hand, with the imminent clinical emergence of the calcimimetic compounds [134, 135].

Many further studies are urgently needed to comprehend the fascinating and highly complex biology of disordered vascular calcification in the setting of chronic renal failure. Vascular calcification in renal patients, like the presence of cardiac valvular calcification in dialysis patients [136], may be a marker of increased cardiovascular risk, well before the actual presence of the calcium has caused a major functional perturbation, perhaps the dialysis patients' equivalent of microalbuminuria in diabetes or hypertension [137]? With our present level of knowledge, which is heavily skewed by a single interventional study, it is premature in our opinion to do more than to pay much greater attention to successful manipulation of plasma phosphate levels by better dialysis techniques, better patient education about diet and phosphate binders, avoidance of marked or prolonged positive calcium balance, calibrating the use of vitamin D more successfully. Further research on the mechanism of action of sevelamer on vascular calcification is urgently needed. Also, in the light of numerous clinical and experimental studies in renal and nonrenal subjects establishing a link between dyslipidemia and propensity to vascular calcification, having a low threshold for the use of statin-based lipid-lowering therapy in a group of patients with the dubious distinction of having the greatest measured major adverse cardiovascular event rates of any reported patient cohorts is, in our opinion, mandatory, unless a large-scale randomized controlled trial shows no benefit.

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